IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of YURA et al., Group Art Unit: 1623

Serial No. 09/937,991 Examiner: Ganapathy Krishnan

Filed: January 23, 2002

For: Functionalized Glycosaminoglycan Polymer and Medical Instruments and Drugs by Using the Same

Docket: 33944

DECLARATION UNDER 37 C.F.R. § 1.132

I, Hirofumi Yura, declare and state:

1. I am a co-inventor of the invention described in patent application Serial No. 09/937,991. I am familiar with chemical reactions of hydrocarbons, in particular, carbohydrates.

2. My educational background includes a Master of Science degree in Chemical Engineering from Tokyo University of Agriculture and Technology.

3. I was employed by Terumo Corporation since April of 1986, and worked as a researcher in R&D division thereof to June of 1995. I joined a project for establishing Netech Inc., from July of 1995, and I am currently a representative of Netech Inc.

4. Claims 1-3 and 6-11 of the present patent application Serial No. 09/937,991 were rejected under U.S.C. § 102(b) as being anticipated by Tay et al (Biomaterials, 1989, Vol.10(1), pp 11-15) and Larsson et al (WO 93/05793). However, I believe that neither Tay et al nor Larsson et al discloses the inventions claimed in the present application, as explained below.

(1) The present application claims a functional polymer having the structure represented by the following formula (1):

$$-(CWX-CYZ)_n- \quad (1)$$

wherein, W denotes a carbohydrate chain including a structure corresponding to at least a portion of the basic skeletal structure of a glycosaminoglycan and comprising 2-50 constituent disaccharide units having an average of at least one sulfate group, X, Y and Z denotes any substituent group including a hydrogen atom, and n denotes the number of repeating units of at least one (Claim 1).

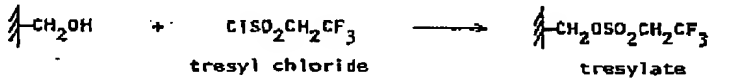
That is to say, the present invention provides a functional polymer, in which each repeating unit constituting the polymer backbone has a carbohydrate chain (W). This structure could be obtained by a homo-polymerization of vinyl-type monomers (CWX=CYZ), each of which includes a carbohydrate chain (W).

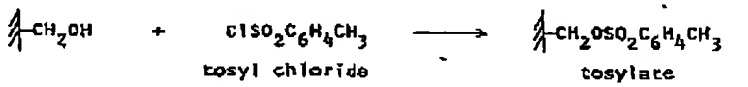
Claims 2, 3 and 11 depend on Claim 1. Claim 7 relates to an agent for preventing reconstriction of a blood vessel comprising the functional polymer of Claim 1, and Claims 8-10 depend on Claim 7. Therefore, the inventions recited in Claims 1-3 and 6-11 include all the limitations recited in Claim 1.

(2) Tay et al discloses, I believe, polyvinylalcohol (PVA) gel, in which only a portion of the OH groups binds to a heparin. However, the Examiner's recognition is that since all the OH groups of the PVA of Tay et al can bind to heparin molecules through activation of OH groups with tresyl chloride, in view of Nillson et al (Biochemical and Biophysical Research Communication, 1981, 102(1) 449-457), the resulting heparin-bound PVA of Tay et al is identical to the functional polymer claimed in the present application.

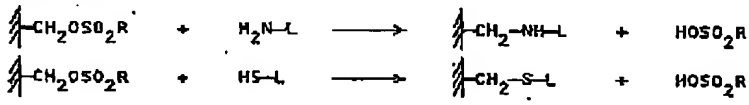
Nilsson et al discloses activation of OH groups with tresylates and coupling of a ligand or enzyme to the activated OH groups through the following mechanisms.

Activation:





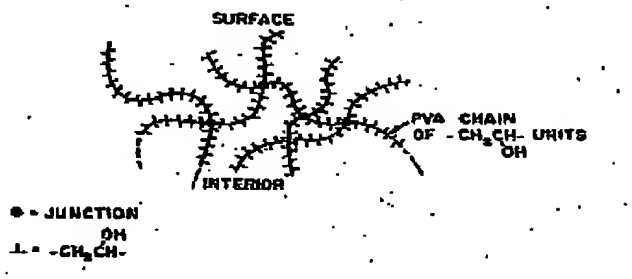
Coupling:



(R = CH_2CF_3 or $C_6H_4CH_3$; L = ligand or enzyme)

According to the above mechanisms, the coupling reactions of the ligands (L) to the activated OH groups are considered as nucleophilic substitutions.

Tay et al uses the process described in Nillson et al for coupling of heparins to OH groups of cross-linked PVA, having the following structure (page 12, Figure 1).



Please note that if all the OH groups could be activated with tresyl groups, all the activated OH groups must be substituted with heparin molecules via nucleophilic substitution in accordance with the above-described mechanisms, in order to obtain the functional polymer as claimed in the present application.

Regarding the nucleophilic substitution in organic chemistry, it has been known for a person of ordinary skill in the art that "steric hindrance" prevents the substitution reaction (please refer to the attached technical publication, "Organic Chemistry", J. W. Suggs, Brown University, NY). As described in Suggs (page 128, Example), a halide at a bridgehead position of bicyclo[2,2,2]octane cannot undergo a nucleophilic substitution (S_N2 reaction) due to "steric effects".

The PVA used in Tay et al is a cross-linked polymer, which inherently creates a three-dimensional network (a specific environment). In particular, in the vicinity of the junctions (●) in the cross-linked network, chemical structures of the polymer molecule are considered as being relatively rigid, and the situation as observed in the bridgehead position of bicyclo[2,2,2]octane tends to occur in such regions.

In fact, referring to the experimental results listed in Table 1 (page 14) of Tay et al, 0.667 to 9.3 mg of heparin was coupled to 8.2 to 56.0 μ M of activated OH groups of PVA. Since the molecular weight of heparin used in Tay et al is about 7000 (page 13, left col. line 5), 0.667 to 9.3 mg correspond to 0.10 to 1.33 μ M. Accordingly, only about 0.9 to 2.4% of the activated OH groups were substituted with heparin molecules

in Tay et al.

Returning to Nillson, the activated OH groups were substituted with various ligands (Concanabalin A, Soybean trypsin inhibitor, Hekisokinase) as listed in Table 2 (page 453). However, amounts of activated OH groups are at most 50% of OH groups in the polymer (agarose). More specifically, the experiments of Nillson et al use several types of "Sepharose" as OH-containing polymeric substances. "Sepharose" is known to be constituted by agarose, which consists of repeating units of D-galactose and 3,6-anhydro-L-galactose (molecular weight of the repeating unit is 342). Since D-galactose contains one OH group but 3,6-anhydro-L-galactose does not contain an OH group, 1g of Sepharose contains 2.92 mmol of OH groups. On the other hand, among the results listed in Table 1 of Nillson et al (page 452), the largest amount of tresyl groups bound to 1g of Sepharose is "1.35 mmol/g" (obtained with "Sepharose CL-4B). This result indicates that only about 46% of OH groups in 1g of Sepharose CL-4B were activated by tresyl groups.

Accordingly, if all of the activated OH groups were substituted with ligands, unreacted OH groups would remain in the resultant polymer. Furthermore, the amount of soybean trypsin inhibitor bound to the polymer did not substantially change if the amount of ligand or coupling time was changed (page 454, Table 3). This means that Nillson et al does not suggest how to increase the amount of ligands bound to the polymer. In contrast, footnote (e) of Table 3 in Nillson et al states that higher specific activity of the ligand (Trypsin) was observed at smaller amounts of the ligand bound to the polymer. Furthermore, Tay et al also describes a substantial decrease of specific activity as the density of binding (of heparin) increases (page 14, right column, lines 5-6). Therefore, from the results shown in Table 1 of Tay et al (page 14), a person of ordinary skill in the art would consider that the amount of heparin bound to PVA must be decreased in order to improve the specific activity. In other words, these descriptions teach away from increasing density of heparins on the PVA chain.

(3) Similarly, the polymers of Larsson et al are prepared by coupling glycosaminoglycan residues to a polymer (such as polylysine). Therefore, it is difficult or almost impossible to incorporate heparins to all the amino groups of the polymer due to steric effects. In addition, the polymers of Larsson et al are also intended to provide antithrombin activity. Therefore, referring to the experimental results of Tay et al, a person of ordinary skill in the art will avoid increasing the density of heparins on the polymer backbone. In fact, Larsson et al describes that

glycosaminoglycan residues should not be located so closely that they will interfere with each other, but neither should they have too wide gaps between them (page 9, lines 8-11). Moreover, Larsson et al also describes that by selecting a polylysine having a molecular weight above 400,000, a synthetic proteoglycan having up to 500 heparin chains per carrier molecule may be prepared (page 14, lines 5-8). Since lysine monomer has a molecular weight of 128, a heparin chain exists per every 6.25 monomer units.

(4) A prior art publication must disclose the claimed invention so that the invention can be carried out by a person of ordinary skill in the art, if the claim is rejected under U.S.C. § 102(b) as being anticipated by that prior art publication (In re LeGrice, 301 F. 2d 929, 133 USPQ 365 (CCPA 1962)).

Accordingly, I believe that neither Tay et al nor Larsson et al discloses the inventions claimed in Claims 1-3 and 6-11 of the present application, and therefore, the present application should not be rejected under U.S.C. § 102(b) over Tay et al (in view of Nillson et al) or Larsson et al.

5. Claim 12 of the present patent application Serial No. 09/937,991 was rejected under U.S.C. § 102(b) as being anticipated by Joh (US Patent No. 4,415,490).

(1) Claim 12 depends on Claim 11 (limiting the polymer main chain of Claim 1 to a vinyl polymer), and recites limitations that the polymer main chain is hydrophobic and the carbohydrate chain is hydrophilic.

(2) Joh discloses non-thrombogenic materials, which are prepared by coupling heparins to the surface of an aldehyde-containing polymer film. The aldehyde-containing polymer film is prepared by surface treating of a polymer film with periodic acid or lead tetra-acetate to cleavage carbon-carbon bonds to give aldehyde groups on the surface of the polymer film. That is to say, Joh only discloses a surface-modification of polymer film, and the resulting non-thrombogenic materials of Joh have heparin structures only on their surfaces.

(3) Accordingly, I believe that Joh does not disclose the invention claimed in Claim 12 of the present application, and therefore, Claim 12 of the present application should not be rejected under U.S.C. § 102(b) over Joh.

6. I hereby declare that all statements and representations made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and representations were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued therefrom.

Dated: 2005, Jan. 20

Signed: Hirofumi Yura

Hirohumi Yura

Organic Chemistry

J. William Suggs, Ph.D.
Associate Professor of Chemistry and Biochemistry
Brown University

BARRON'S

All inquiries should be addressed to:
Barron's Educational Series, Inc.
250 Wireless Boulevard
Hauppauge, New York 11788
http://www.barronseduc.com

International Standard Book No. 0-7641-1925-7

Library of Congress Catalog Card No. 2002018597

Library of Congress Cataloging-in-Publication Data

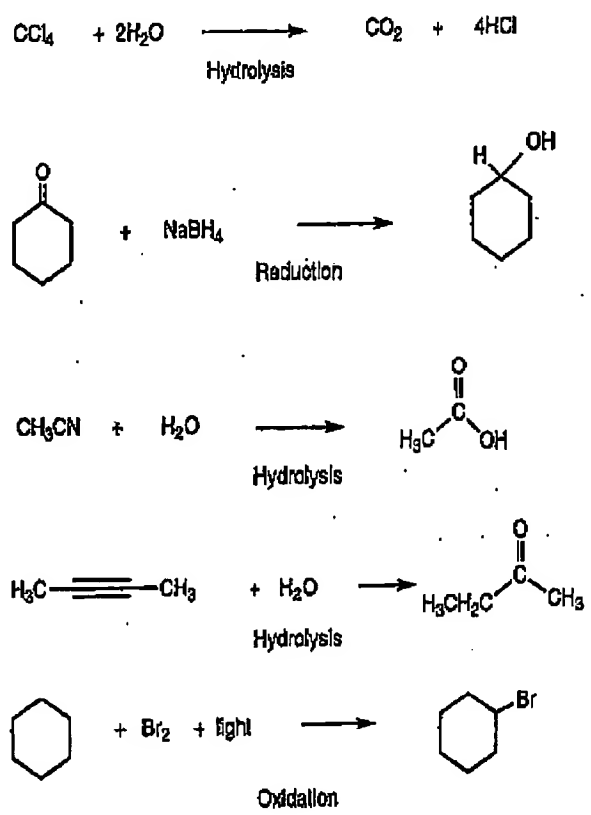
Suggs, J. William.
Organic chemistry / J. William Suggs.
p. cm.—(Barron's college review series)
Includes index.
ISBN 0-7641-1925-7
1. Chemistry, Organic—Outlines, syllabi, etc. I. Title.
II. College review series.
QD256.5 .S84 2002
547'.076—dc21

2002018597

PRINTED IN THE UNITED STATES OF AMERICA
9 8 7 6 5 4 3 2 1

CONTENTS

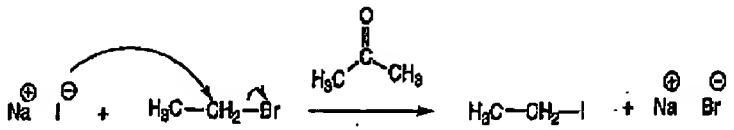
Example The following are examples of oxidation, reduction, and hydrolysis reactions.



8
NUCLEOPHILIC SUBSTITUTION

NUCLEOPHILIC SUBSTITUTION REACTIONS

The first reaction that we will examine is the nucleophilic substitution reaction. An example is the reaction of sodium iodide, a source of I^-, with ethyl bromide in the solvent acetone to give ethyl iodide and sodium bromide:



Here the iodide anion is the nucleophile, the carbon bonded to the halide is the electrophile, and the group being substituted or displaced, the **leaving group**, is Br^-. Normally, in writing organic reactions the solvent and any other reaction conditions (such as the temperature, a catalyst, and so on) are drawn above or below the reaction arrow. For every nucleophilic substitution reaction there are a nucleophile, an electrophile, and a leaving group.

To understand this reaction, we must deduce its mechanism. Clues to the mechanism will come from a study of the reaction's kinetics, its stereochemistry, and how changes in the nucleophile, electrophile, leaving group, and reaction conditions affect the reaction. Organic chemists can make literally millions of molecules capable of undergoing a nucleophilic substitution reaction. Our goal is to understand all the general factors that influence this reaction so that we can decide if a specific nucleophilic substitution reaction will proceed as written.

Kinetics of Nucleophilic Substitution

Ultimately, science is based on observables. A feature of a nucleophilic substitution reaction often easy to observe is the rate at which the starting materials are used up or the products are formed. For the ethyl bromide reaction shown above, the solvent acetone was chosen because NaI is soluble in acetone but NaBr is not. Thus, one could follow the rate of the reaction by

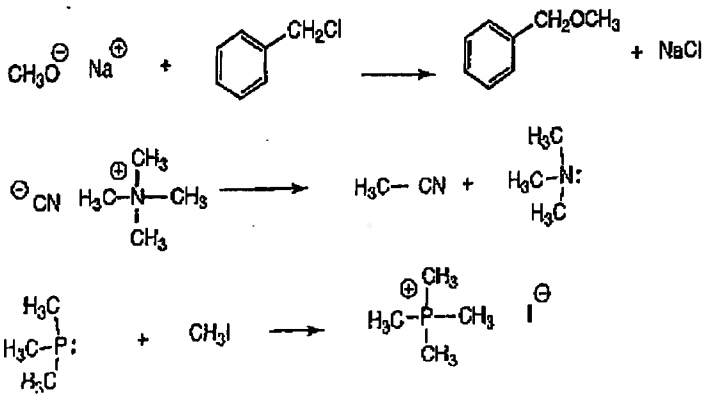
collecting and weighing the amount of NaBr precipitate formed at specific times. Because the concentration of one of the products of this reaction is made to equal zero by this solvent trick, the equilibrium of the reaction lies completely to the right. By **Le Châtelier's principle**, this reaction is made *irreversible*.

Chemists now have sophisticated instruments to measure concentration changes of organic molecules in solution, so we do not have to weigh salts as they precipitate out of solution. When the rate at which the concentration of ethyl bromide decreases is measured for the nucleophilic substitution reaction of EtBr with NaI, the **rate law** found is

$$\frac{-d[H_3C-CH_2Br]}{dt} = k[H_3C-CH_2Br][NaI]$$

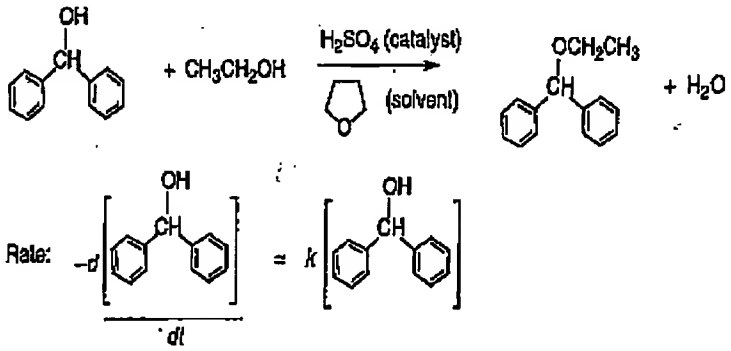
The rate at which the concentration of the electrophile decreases (i.e., the rate at which it is turned into products) is a function of the concentration of the electrophile and the concentration of the nucleophile. Doubling the concentration of EtBr forms product at twice the previous rate. Doubling the concentrations of both EtBr and NaI increases the rate by a factor of 4. The reaction is kinetically **second order**, which is the sum of the exponents of the concentration terms on the right hand side of the equation (1 + 1). The factor k is the **second-order rate constant** and has the units $M^{-1}s^{-1}$. It is a constant only for a specific set of reaction conditions. It increases with temperature and can increase or decrease with a change in solvent.

The reaction of EtBr with NaI is an example of an **S_N2 reaction**, which stands for substitution, nucleophilic, kinetically second order. Any time we use the term S_N2 to describe a reaction, its kinetics are second order. Some other examples of S_N2 reactions are shown below. The nucleophile appears first in each case.



Notice that a neutral nucleophile and a neutral electrophile react to give a salt, as in the third reaction, and that the anion and cation of an organic salt can react to give two neutral molecules, as seen for the second reaction.

Not all nucleophilic substitution reactions are kinetically second order. For example, when the kinetics of the following acid-catalyzed reaction were studied, the rate law was found to be first order:



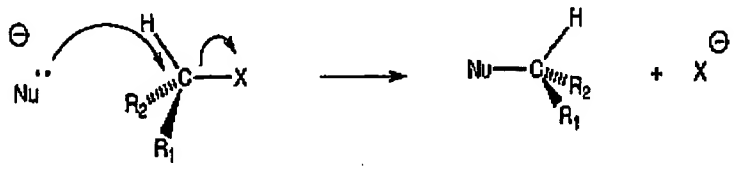
It so happens that many nucleophilic substitution reactions are kinetically **first order**; that is, the rate depends only on the concentration of the electrophile. The concentration of the nucleophile is not part of the rate law, and changing its concentration has no effect on the rate. For a first-order reaction, the rate constant has the units s^{-1}. The term **S_N1** is used to describe substitution, nucleophilic, kinetically first-order reactions.

The species present as concentration terms in a rate law are the species present in the **rate-determining step** of the reaction mechanism. The rate-determining step of a reaction is like the bottleneck of a reaction. An analogy is often made with the flow of water through a system of pipes. If pipes of different diameters are joined together, the rate at which water flows through the system depends on how fast it can flow through the smallest-diameter pipe. The smallest-diameter pipe determines the rate of flow just like the rate-determining step of a reaction determines the rate that molecules move through a multistep reaction.

Since S_N2 and S_N1 reactions have the nucleophile and electrophile, or only the electrophile, respectively, present at their rate-determining steps, they must have different mechanisms. In fact, the differences are so numerous that they are best considered separately, although they are both nucleophilic substitution reactions. The S_N2 reaction is somewhat simpler mechanistically and is treated first.

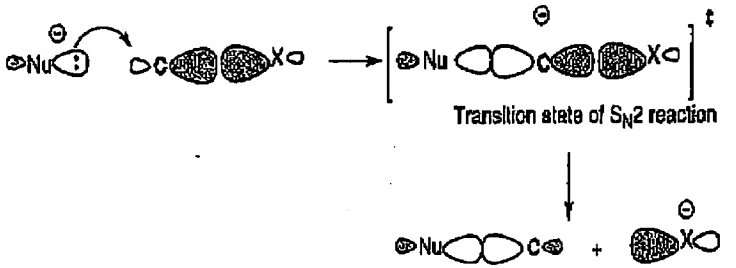
Stereochemistry of the S_N2 Reaction

Knowing the stereochemistry of a reaction limits the possible mechanisms one can put forward for a given reaction. For an S_N2 reaction, there is a very precise relationship between the stereochemistry of the starting material and the product. An S_N2 reaction proceeds via **inversion** at the electrophilic carbon:

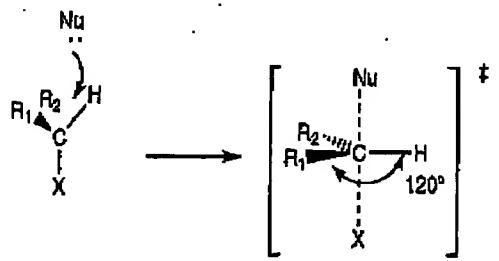


Inversion is a shape change for the electrophilic carbon undergoing an S_N2 reaction. It is exactly like what happens to an umbrella in a high wind. The nucleophile comes in on a trajectory 180° opposite the leaving group, staging a **backside attack.** The other groups move toward the opposite side of the carbon, while the bond to the leaving group breaks. Inversion was experimentally demonstrated by the chemist Paul Walden, so it is sometimes called **Walden inversion.** As the orbital diagram below indicates, in the transition state for an S_N2 reaction, the nucleophile, electrophilic carbon, and leaving group are *colinear.*

Orbital changes:



During the discussion of hybrid orbitals, the presence of the tail of the sp^3 orbital was mentioned. It is this part of the orbital that initially interacts with the incoming nucleophile. When the transition state structure for an S_N2 reaction is reached, the orbital has come to resemble an unhybridized p orbital. The geometry of the transition state is called **trigonal bipyramidal:**



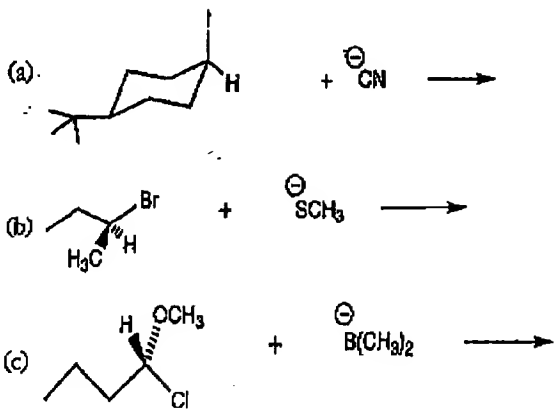
Trigonal bipyramidal transition state for an S_N2 reaction

The octet rule is not violated in the transition state because the bond to the nucleophile is only partly formed and the bond to the leaving group is partly broken.

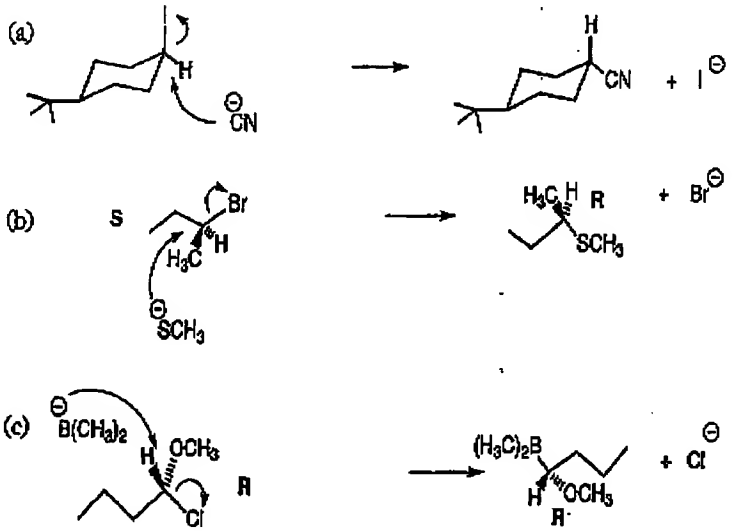
For chiral electrophiles, one can assign the absolute configuration of the carbon undergoing reaction as R or S. Often the R isomer gives the S product, and vice versa. However, the R and S relationship is independent of the inversion geometry and depends only on the atomic weight of the nucleophile and the leaving group. In addition, carbons with no stereogenic centers, even molecules like CH_3I, still undergo the geometric shape change of inversion.

EXERCISES

Sketch the mechanism of the following S_N2 reactions using curved arrows and showing inversion at a carbon. For chiral molecules, assign R and S to all stereogenic centers.



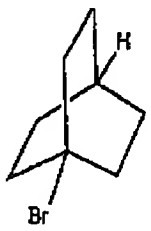
Solutions



Note: This reaction shows that an R isomer can give an R product along with inversion. These particular reagents might not react this way in real life.

Example Explain why a halide at a bridgehead position cannot undergo an S_N2 reaction.

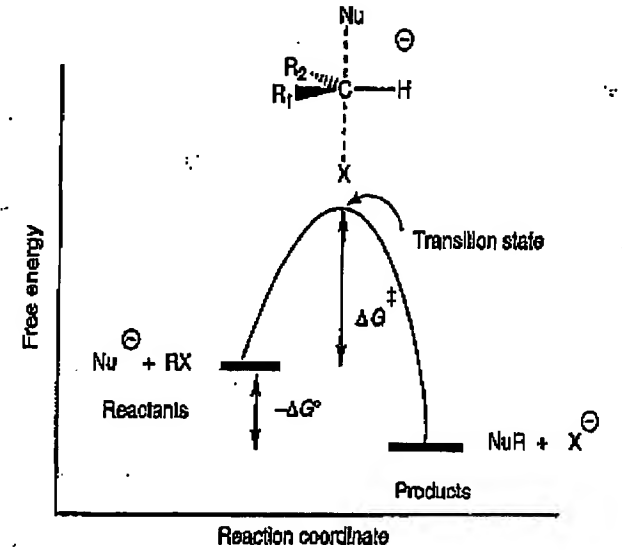
Solution: The answer is in the geometry of the S_N2 reaction. A bridgehead halide, such as the bicyclo[2.2.2]octane molecule



has a another bridgehead carbon directly behind it. No nucleophile is small enough to occupy the space behind the C—Br bond. Thus, the S_N2 transition state geometry cannot be attained. This is an example **steric effects** preventing an S_N2 reaction.

Reaction Coordinate Diagram for the S_N2 Reaction

During the course of a reaction, the energy of the system changes as bonds break and re-form. A **reaction coordinate diagram** plots the total free energy of the reaction vs the reaction coordinate, which measure the progress of the reaction. The reactants are placed on the left side of the diagram, and the products on the right. The reaction coordinate diagram for an S_N2 reaction is given here.



In an S_N2 reaction, there is a single **transition state** between the reactants and products. The transition state represents the maximum energy point between two energy minima. It exists for only one molecular vibration, and any change, either one that leads back to reactants or one that leads to products, is stabilizing. The energy difference, $\Delta G^{\ddagger}$, between the reactants and the transition state is the **energy of activation**. This energy determines the rate of the reaction. For this particular diagram, the products are lower in energy than the reactants by $-\Delta G^{0}$, so the reaction is **exergonic**. The equilibrium lies to the right. If the products were higher in energy than the reactants, the equilibrium would lie to the right, the free energy change would be positive, and the reaction would be **endergonic**.

A reaction coordinate diagram is a graphical depiction of the information in the rate law. The rate-determining step for an S_N2 reaction is formation of the transition state. In general, the step in which the transition state is highest in overall energy is the rate-determining step. Since the rate law is bimolecular, both the nucleophile and the electrophile must be present in the transition state of the rate-determining step.